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Enzootics in the New York Aquarium Caused by *Cryptocaryon irritans* Brown, 1951 (= *Ichthyophthirius marinus* Sikama, 1961), a Histophagous Ciliate in the Skin, Eyes and Gills of Marine Fishes.

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(Plates I-VII)

Introduction

A PARASITIC holotrichous ciliate resembling *Ichthyophthirius multifilis* Fouquet, the well-known "Ich" of freshwater fishes, was first reported by Sikama in 1937 from more than 45 species of marine fishes dying in aquaria from several localities of the Institute for Fisheries of the Tokyo Imperial University, especially in the Aiti Prefecture, Japan. He (1938) referred to the disease as "Weisspunktchenkrankheit," or "white spot disease" of marine fishes, suggesting its similarity to the common "Ich." However, Sikama (1960, 1961) recognized that the ciliate differed distinctly from the freshwater species in body shape, in nuclear features, and in its specificity for marine fishes. For these and other reasons he (1961) named the parasite *Ichthyophthirius marinus*. However, Sikama and, more recently, Kudo (1966) were unaware that the ciliate had been named *Cryptocaryon irritans* by Eleanor Brown in 1951, based on preliminary description of the ciliate obtained from marine fishes in the Aquarium of the Zoological Society of London. A more complete description of the meganuclear cycle was given by her in 1963. *A priori, Ichthyophthirius marinus* Sikama, 1961, is a synonym of *Cryptocaryon irritans* Brown, 1951.

The present contribution deals with observations on the susceptibility and pathogenesis of marine fishes in the New York Aquarium to *Cryptocaryon irritans* together with additional information on its life history, morphology and cytology.

Material and Methods

The parasites were first observed in the New York Aquarium in 1958, reaching enzootic proportions in 1964. Table 1 lists the susceptible host species in the Aquarium's collection for 1964-1966.

Both skin and gill infected tissues were fixed in Bouin's and in 10% neutral formalin; the sections were stained with Harris' hematoxylin-eosin and with Heidenhain's Iron-hematoxylin with and without eosin. Freed ciliates were also fixed in formalin and stained *in toto* with H-E; some were allowed to dry on slides and treated with 2% $AgNO_3$ and reduced with U-V to demonstrate the silver-line system.

The activity of the motile form, its encystment and reproduction, was followed continuously for many hours. For this purpose, the ciliates were removed by shaking infected gills in sea water, centrifuged and washed five times with millipore-filtered sea water. The organisms were then distributed to several syracuse dishes with filtered sea water and observed both in the dishes and on wet-mount preparations by light, phase and interference microscopy.

Some of the fish were simultaneously infected with *Oodinium ocellatum*, a parasitic dinoflagellate to which these fish are also susceptible. These were present in small numbers in the living preparations of the ciliates and were used for comparing time of encystment and division in both species.

Life History

The life history of *Cryptocaryon irritans* is similar in many respects to *Ichthyophthirius multifilis*. The stages in the cycle are as follows: (1) the trophont, or parasitic (feeding) stage, in the skin and gills (Pl. figs. 1-7); (2) the tomont, or encysted stage off the host in which reproduction takes place to produce (Pl. figs. 8-13); (3) tomites, or small, free-swimming, non-feeding ciliates that eventually infect the same or other susceptible hosts (Pl. fig. 16).

The living trophonts of *Cryptocaryon* vary in size from 48 x 27 to 450 x 350 microns. "Older" individuals are usually filled with numerous, densely packed granules, obscuring the meganucleus. No contractile vacuoles were noted. In young parasitic forms, ingested cellular debris and blood cells are often seen. The ciliates are highly plastic, changing their shape constantly as they move about in the skin and gills of the host (Pl. figs. 1-3). However, they all tend to assume a more or less oval shape when forced to become free-swimming (Pl. fig. 2), extending or retracting the buccal end when they encounter other individuals or pieces of debris (Pl. fig. 3). Eventually, motility slows down and the cilia are gradually absorbed, followed by the development of the cyst membranes (Pl. fig. 8).

The factors responsible for the transition of the trophont to the tomont are not known. This change, i.e. cessation of active feeding and dropping off the host, is not due to a sudden lowering of the temperature, a factor that is well known for *Ichthyopthirius*.

Regardless of size, once the trophont leaves the host for whatever cause (e.g. death of the host), the ciliate slowly becomes encysted and undergoes its reproductive phase. The following events were observed in washed ciliates maintained in filtered sea water in syracuse dishes and in wet-mount preparations at room temperature: 10-15% of the ciliates encysted in 4 hours; these cysts varied in size from 94.5 x 170 microns to 441 x 252 microns. 100% encystment occurred within 20 hours; during this time *Oodinium* was in the 4-cell stage (Pl. fig. 9). Within 24 hours all the encysted forms which were uniformly opaque showed numerous peripheral vacuoles (Pl. fig. 9), similar to those described by Brown (1963). No obvious changes were noted in the cysts for the next 24 hours, but *Oodinium* had divided to the 32-cell stage. At 110 hours, the dinoflagellates were free-swimming while some of the tomonts showed various stages of division. Division is unequal and polar, which, according to Brown (1963), may be a form of budding rather than simple fission (Pl. fig. 10). The polar cap divides further to form a group of cells (Pl. fig. 11); later division occurs in the residual mass, eventually giving rise to a number of similar sized ciliates within the cyst (Pl. fig. 12).

In our studies, the tomites started to emerge from various cysts from the 6th to the 9th day, with most of them emerging on the 8th day. The number of tomites produced depends on the size of the tomont; some of the largest individuals may form 200 or more free-swimming forms (Pl. fig. 14). Also, the time of emergence is not related to cyst size. For example, some of the smallest tomonts, encysted at the same time as the largest, developed tomites at the same time or later than the largest individuals.

The fully developed tomites show motility within the cyst, and appear to emerge from a small opening (or openings) on one side of the cyst wall as thin, flattened forms (Pl. fig. 13). In no case was the cyst completely ruptured naturally, nor was there any evidence of complete cyst dissolution indicative of enzyme action (Pl. fig. 15).

The newly-emerged tomites are pear-shaped and on the average measure 56.5 x 35 microns. The buccal membranelles and the meganucleus with its four distinct spherical bodies are well developed (Pl. fig. 16). The young ciliates at first swim slowly nearby and then suddenly increase their swimming activity, darting vigorously away from the parent cyst. The evidence indicates that they are phototropic. The tomites remain free-swimming for a relatively short period; no accurate time was determined but it appears to be less than 24 hours.

Cytology

Trophont (Pl. figs. 1-7). The exact number of kineties could not be determined in our silver nitrate treated preparations. However, the elements are parallel, terminating at the oral region (Pl. fig. 4). There is a well-developed buccal cavity with a protrusible apparatus, the detail structure of which was not too clear in our present preparations. Brown (1963) reports two membranes in this complex, a large, stiff protrusible membrane on the left wall overlying a small membrane on the right wall.

Serial sections of stained gill preparations clearly show the cytological details of the meganucleus in several individuals of various sizes and were similar to those seen by Brown in preparations stained with Heidenhain's hematoxylin. The meganucleus consists of four spherical bodies linked into a U- or crescent-shaped structure (Pl. fig. 5). There is a well-defined nuclear membrane, a network of chromatin, smaller non-chromatin bodies and numerous densely staining, basophilic, spherical-shaped bodies in vacuole-like areas (Pl. figs. 6 & 7). The number of these bodies varies with the size of the nucleus,

TABLE 1. NEW YORK AQUARIUM HOST LIST FOR *Cryptocaryon irritans* 1964-1966

SCIENTIFIC NAME	COMMON NAME	NUMBER DEAD	LOCALITY
HOLOCENTRIDAE			
Holocentrus ascenionis	Squirrelfish	5	Atlantic
SERRANIDAE			
Paralabrax nebulifer	Sand Bass	1	Indo-Pacific
Grammistes sexlineatus	Golden striped Bass or Grouper	1	Indo-Pacific
LUTIANIDAE			
Lutianus griseus	Gray Snapper	1	Atlantic
HAEMULIDAE			
Orthopristis chrysopterus	Pigfish	1	Atlantic
Anisotremus virginicus	Porkfish	1	Atlantic
SPARIDAE			
Stenostomus chrysops	Northern Porgy	1	Atlantic
SCIAENIDAE			
Eques lanceolatus	Ribbonfish	1	Atlantic
POMACENTRIDAE			
Dascyllus auranus	White-tailed Puller	1	Indo-Pacific
LABRIDAE			
Labroides phthirophagus	Cleaning Wrasse	1	Indo-Pacific
Lachnolaimus maximus	Hogfish	1	Atlantic
CHAETONDONTIDAE			
Pomacanthus para	French Angelfish	1	Atlantic
Angelichthys bermudiensis	Bermudian Blue Angelfish	1	Atlantic
Pomacanthus semicirculatus	Korean Angelfish	1	Indo-Pacific
Pomacanthus imperator	Imperial Angelfish	1	Indo-Pacific
ACANTHURIDAE			
Acanthurus coeruleus	Blue Tang	1	Atlantic
Acanthurus achilles	Achilles Tang	1	Indo-Pacific
BALISTIDAE			
Balistes vetula	Queen Triggerfish	2	Atlantic
MONACANTHIDAE			
Alutera schoepfi	Orange Filefish	1	Atlantic
OSTRACIIDAE			
Ostracion tuberculata	Ocellated Boxfish	1	Indo-Pacific
Lactophrys quadricornis	Cowfish	1	Atlantic
Lactophrys triqueter	Smooth Trunkfish	1	Atlantic
DIODONTIDAE			
Chilomycterus schoepfi	Spiny Boxfish	1	Atlantic
Diodon hystrix	Porcupinefish	2	Atlantic
SCORPAENIDAE			
Pterois volitans	Lionfish	1	Indo-Pacific
TRIGLIDAE			
Prionotus evolans	Sea Robin	1	Atlantic
BATRACHOIDAE			
Opsanus tau	Toadfish	12	Atlantic

evidently increasing in number with the growth of the organism. It should be noted here that the increase in total size of the individuals is not entirely cytoplasmic but most of it is due to swelling or distention from ingestion of food material.

In addition to the meganucleus, a number of micronuclei are usually present, invariably in the interphase stage. Sikama (1938) reported 5-6 micronuclei, while Brown found 4-7 in tomites, with this number persisting in the young trophont. In any event, micronuclei are also clearly visible in some of our preparations of trophonts of various dimensions (Pl. figs. 7 & 17).

Tomont (Pl. figs. 8-15). No sections were made of the tomonts. The number of membranes forming the cyst was not clearly evident. Most of the tomonts showed at least 4 membranes; Sikama (1961) suggests that the cyst wall is formed by the 10 layers of very thin lamellae, at least 6 forming the outer cyst wall. Occasionally, the membranes are abnormally formed.

Tomites (Pl. figs. 16 & 17). The pear-shaped tomites clearly show the four spherical bodies making up the nucleus, occupying at least two-thirds of the posterior end of the body. The basophilic inclusions are not formed at this stage. The number and arrangement of the kineties was not established but the cilia as well as the buccal membranes are well developed. The young trophonts stained with hematoxylin show the complex structure of the meganucleus and the micronuclei (Pl. fig. 17).

Pathogenesis

Fishes maintain an immunity by premunition, i.e. by the presence of the parasite but without evidence of pathogenic lesions. The factors responsible for the pathogenicity of *Cryptocaryon* or *Ichthyophthirius* are still not known. Enzootics in freshwater fishes caused by *Ichthyophthirius* invariably starts with a sudden drop in temperature. The inocuous trophont drops off the host, settles to the bottom and becomes transformed into the tomont, which eventually gives rise to hundreds of astomatous, non-feeding, free-swimming ciliates, which swarm towards the same or different host. Once contact is made, the ciliates develop a buccal apparatus and vigorously burrow into the skin epithelium, causing the papules characteristic of Ichthyophthiriasis. As the trophonts grow at the expense of the host, they become visible to the naked eye and give rise to the typical white spot lesions well known to aquarist and fish specialists.

The initiation of the cycle in *Cryptocaryon*, however, is not dependent on the drop in temperature, even though the end result is similar. The lesions on the skin of marine fishes do not necessarily appear as white spots but rather as numerous minute, grayish vesicles. Like *Ichthyophthirius,* the tomites swarm towards susceptible host invading the epithelium of the gills and skin.

The irritating effects of the parasites, which may be a mechanical process or caused by chemical substances produced by the ciliates, is manifested by the excessive production of mucous on the body and gills. The petechial lesions on the body and in the gills may be foci for secondary infections with non-specific *Pseudomonas.* Heavy infections invariably result in death of the host. The lesions on the gills are more dramatic. The parasites invade the epithelial lining of the lamellae, causing considerable erosion of tissue and excessive effusion (Pl. fig. 1). In some instances, the parasites also invade the eyes, frequently causing blindness.

An effective treatment for Cryptocaryoniasis was developed by Dr. Morris Baslow, formerly of our staff, but the material should be used with extreme caution because of its toxicity. Diseased fish are treated with 1 cc. of the following stock solution added to each 25 gallons (U.S.) of sea water: Formalin, 100 cc.; cupric acetate, 8 grams; *Tris,* 92 grams. The solution will have a final adjustment of pH at 7.5 at 24°C. Usually, a single treatment is sufficient but can be repeated if needed.

A simpler, less toxic but still an effective remedy, at least for treating skin and eye infections in *Pterois volitans,* is to add 0.15-0.2 ppm of copper sulfate–citric acid solution to the treatment tank and enough methylene blue (1 cc. of 1% solution per 2.5 gallons of water) to produce a clear blue color. The treatment should be repeated at intervals of 5 days for at least 15 days.

Discussion

There is little doubt that the ciliate responsible for certain enzootics in marine fishes kept at "tropical" temperatures (22-25°C) in the New York Aquarium is the same as that reported by Sikama (1937, 1938, 1960, 1961), Brown (1950, 1951), de Graaf (1962), and as that seen by Laird (1965) in Singapore fishes in 1955. The ecto-parasite was first reported as *Ichthyophthirius multifilis* by Sikama in 1937 in a Japanese paper which was translated into German in 1938. In 1961, he redescribed and named the organism *Ichthyophthirius marinus,* recognizing certain morphological and cytological details that distinguished it from the freshwater species. The description by Brown (1951, 1963), by Sikama (1961) and in the present contribution definitely establish *Cryptocaryon irritans* as a distinct genus and species closely related to *Ichthyophthirius multifilis.* The ciliate, according to Corliss (1961), belongs to the Order Hymenostomatida, Suborder Tetrahymenina and Family Ophyroglenidae (=Ichthyophthiriidae).

With the increased importation of Hawaiian and Indo-Pacific fishes by various aquariums in the world since the Second World War, *Cryptocaryon irritans* has now become established as an important disease-producing entity in marine fishes kept in captivity at temperatures ranging from 20 to 26°C. Once established, the parasites show very little host specificity, as can be judged from the host list in Table 1, which shows that fishes affected in the New York Aquarium include both North and South Atlantic species.

The parallelism of Cryptocaryoniasis to Ichthyophthiriasis of freshwater fishes is striking, a phenomenon also seen with other ciliates. For example, many marine fishes may also become infected with *Trichodina* or *Trichodina*-like spp. (Pl. fig. 18) and/or with *Chilodonella*-like species (Pl. figs. 19 & 20); the latter ciliate, although known to occur in salt or brackish waters, either free-living or as ecto-commensal on amphipods, has not been previously reported as a parasite of marines fishes.

Summary

1. *Cryptocaryon irritans* Brown, 1951 (= *Ichthyophthirius marinus,* Sikama, 1961) is reported from the skin, gills and eyes of Indo-Pacific and Atlantic fishes in the New York Aquarium.

2. The life history, cytology, pathogenesis and treatment are described.

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EXPLANATION OF PLATES

Plate I

Fig. 1. The gills of an Atlantic squirrelfish, *Holocentrus ascensionis*, infected with *Cryptocaryon irritans*, a marine hymenostomatid ciliate. Note the various sizes and shapes of the parasite and excessive production of mucous. In extreme infections, the gill epithelium is completely denuded. 50 ×.

Fig. 2. Free-swimming trophont with characteristic shape and extended buccal apparatus. 600 ×.

Plate II

Fig. 3. Another ciliate with buccal end slightly retracted. 600 ×.
[Figs. 2 & 3 show relatively large trophonts (about 135 × 115 microns) filled with food material which is responsible for the distention. The food material consists of cells, cellular debris and blood.]

Fig. 4. Silver nitrate preparation of a larger trophont; note the parallel arrangement of the kineties, terminating in a ring around the buccal apparatus. 300 ×.

Plate III

Fig. 5. Photograph of a fairly advanced stage of development of *Cryptocaryon irritans* showing the characteristic beaded arrangement of the nucleus. Haematoxylin stained preparation made by Dr. Marshall Laird in 1955 from Singapore fishes. About 1200 ×.

Fig. 6. Section of the gills of scup, *Stenotomus chrysops*, from the North Atlantic; showing ciliate cut at the level of the nucleus. According to Dr. Eleanor Brown (1963), the true chromatin is the coarse network; small granules and the larger inclusions in vacuoles, although basophilic are Feulgen-negative. 1350 ×.

Plate IV

Fig. 7. Another section of the gills seen in fig. 6, showing the parasitic ciliates within the gill epithelium. The darkly staining spherical bodies are probably micronuclei. 600 ×.

Fig. 8. Tomonts, or encysted stage, showing the variability in size, shape and structure of the cyst wall; all are viable cysts. 150 ×.

Fig. 9. Details of early division are obscured because of the dense inclusions. The initation of division is indicated by the development of cytoplasmic vacuoles, shown as light areas in this photo 24 hours after encystment. *Oodinium*, a parasitic dinoflagellate that also reproduces in the encysted stage is in the 4-cell stage. 150 ×.

Plate V

Fig. 10. Cellular division, which is unequal, begins at one end, shown here as a polar cap. Note the elongate shape of the tomont. 150 ×.

Fig. 11. The polar cap divided further to form a group of cells. Note the spherical shape of the tomont and difference in size. 150 ×.

Fig. 12. Later division occurs in the residual mass, giving rise to a number of similar sized cells within the cyst. 150 ×.

Fig. 13. Fully developed tomites show motility within the cyst; they appear to emerge from a small opening (or openings) on one side of the cyst wall. 150 ×.

Fig. 14. Cyst ruptured by mechanical pressure to show the numerous tomites. 50 ×.

Plate VI

Fig. 15. Cyst or tomont with a few tomites still present, indicating that the cyst wall is not ruptured or dissolved in order to release the free-swimming stage. 150 ×.

Fig. 16. One of numerous pear-shaped newly emerged free-living tomite showing the typical spherical bodies making up the nucleus; the buccal structure is well developed. 600 ×.

Fig. 17. A very young trophont showing the four spherical bodies that make up the meganucleus and three prominent micronuclei. Delafield hematoxylin stained preparation made by Dr. Marshall Laird in 1955, from Singapore fishes. 600 ×.

Fig. 18. *Trichodina*-like sp. on gills of black sea bass *(Centropristes striatus)* 300 ×.

Plate VII

Fig. 19. *Chilodonella*-like sp. on gills of rainbow parrotfish *(Scarus guacamaia)*. 300 ×.

Fig. 20. Details of *Chilodonella*-like sp. Note typical oral basket membranelle. Hematoxylin-Eosin. 1350 ×.